AWARD NUMBER: W81XWH-14-1-0171

TITLE: YY1 Control of AID-Dependent Lymphomagenesis

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REPORT DATE: July 2015

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
July 2015	Annual	1Jul2014 - 30Jun2015

4. TITLE AND SUBTITLE

YY1 Control of AID-Dependent Lymphomagenesis

5a. CONTRACT NUMBER
W81XWH-14-1-0171

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

We hypothesize that YY1 levels control AID nuclear accumulation, AID mutation rates, and subsequent AID-mediated B cell lymphomagenesis. We are testing this hypothesis by exploring the impact of YY1 overexpression, or deletion, in mouse lines that spontaneously develop AID-dependent B cell lymphoma. In the initial granting period this year, we have bred the mice that spontaneously develop B cell lymphoma and have initiated YY1-overexpression studies. Our results are still preliminary but suggest that overexpression of YY1 leads to higher mortality. Second, we have bred the yy1 f/f and gamma1-CRE alleles onto the background of the mice that spontaneously develop AID-dependent B cell lymphoma. These mice will enable us to test in the coming year if YY1 knock-out reduces B cell lymphoma. Finally, we have demonstrated that knock-out of YY1 results in reduced AID-mediated mutagenesis, supporting our main hypothesis. In the coming year we anticipate being able to complete experiments to test the role of YY1 in controlling AID-dependent lymphoma.

15. SUBJECT TERMS
Nothing Listed

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	10	19b. TELEPHONE NUMBER *(include area code)*

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1. INTRODUCTION

Approximately 40% of all B cell lymphomas are derived from germinal center B cells and nearly half of patients with germinal center-derived diffuse large B cell lymphoma (DLBCL) are refractory to standard treatments, or undergo recurrences and have a very low chance of long-term survival. Activation induced cytidine deaminase (AID) is the mutagenic enzyme directly involved in germinal center B cell lymphomagenesis. Any factor that elevates nuclear AID levels will increase its mutagenic activity, thereby increasing the risk of lymphoma. Notably, we serendipitously found that transcription factor YY1 physically interacts with AID, promotes its stabilization, increases nuclear AID levels, and enhances AID function. Thus, our findings represent a new paradigm for control of AID function and B cell lymphomagenesis. We hypothesize that YY1 stabilization of nuclear AID directly contributes to B cell lymphomagenesis. We will test this hypothesis by modulating YY1 expression a mouse model, λmyc IμHABCL6, which spontaneously develops both AID-dependent and AID-independent B cell lymphomas. We will test our hypothesis in a powerful and innovative bone marrow transplantation model in which bone marrow from λmyc IμHABCL6 mice is transduced with YY1-expressing retrovirus or vector control, then injected into recipient mice to assess the ability of YY1 to impact development of B cell lymphoma. Although YY1 is frequently overexpressed in B cell lymphomas, it is not yet clear if its expression is necessary for generation of germinal center-derived B cell lymphoma. We will assess the requirement for YY1 in lymphomagenesis by conditional deletion of the *yy1* gene in germinal center B cells using γ1-CRE mice.

2. KEYWORDS

Yin-Yang 1 (YY1), B Cell Lymphoma, Activation Induced Cytidine Deaminase (AID)

3. ACCOMPLISHMENTS

What were the major goals of the project?

Task 1. Does YY1 over-expression lead to increased lymphomagenesis or disease progression?

Our plan is to test our hypothesis that YY1 over-expression stabilizes nuclear AID, thereby promoting off target site mutations that drive B cell lymphomagenesis in a powerful and innovative bone marrow transplantation model in which bone marrow from λmyc IμHABCL6 mice is transduced with YY1-expressing retrovirus or vector control, and injected into secondary recipient mice.

1a. Isolate bone marrow from λmyc IμHABCL6 mice, transduce with YY1 retrovirus or vector control, inject into secondary recipient mice.

Time frame: months 1-12. 50% completed

1b. Evaluate tumor latency, tumor development by histology and pathology. Beginning 3 months after transduction and each month thereafter for 10 months, three mice from each group will be sacrificed for evaluation of tumor development. A complete autopsy will be performed on each YY1 overexpressing and vector control mouse. Routine hematoxylin and eosin (H&E) stained slides of spleen, liver, lymph nodes, bone marrow, neoplastic tissue and any other tissues that appear suspect will be reviewed histologically

by a board certified veterinary pathologist (Dr. Amy Durham). Germinal center phenotype will be initially assessed by staining for PNA and CD95.
Time Frame: months 4-18. 20% completed

1c. Molecular profiling of tumors. Neoplasms will be isolated surgically and evaluated molecularly for mutations indicative of germinal center phenotype and by RNA microarray analyses for lymphoma subtyping. Mutations within Ig V and switch region DNA sequences will determined by PCR amplification, cloning, and DNA sequencing. Mutations will also be assessed within the BCL6, DC83, Pim1 genes that are frequent targets of AID off-target mutations, with B2m, Ltb, Taci, Whsc1, H2Ea, A20 genes serving as negative controls. Translocations between c-myc and the IgH locus, or the miR-142 gene will be measured by PCR followed by southern blotting. RNA prepared from tumors will be subjected to microarray analyses using the Penn Vet microarray core facility. In particular we will be assaying for transcript profiles consistent with a DLBCL phenotype (ABC and GC subtypes) and germinal center origin to determine if the observed tumors yield pre-germinal center, germinal center, or post-germinal center signatures as well as ABC and GC phenotypes.
Time Frame: months 6 to 24. 0% completed

1d. Evaluation of tumor aggressiveness. Proliferation potential will be assessed by growing splenic tumor cells ex vivo in the presence of LPS and measuring proliferation using CSFE staining. Tumor cells will be injected into sublethally irradiated (3 Gy) Rag -/- mice and time to tumor development and lethality will be measured. Proliferation will also be measured by injecting BrdU into mice, isolating spleens, and staining with anti-BrdU, as well as the proliferation marker Ki-67. Apoptosis will be assessed by TUNEL staining.
Time Frame: months 6 to 24. 0% completed

Task 2. Is YY1 necessary for B cell lymphomagenesis or disease progression?
We plan to assess the requirement for YY1 in lymphomagenesis using an innovative model in which YY1 expression is conditionally deleted from germinal center B cells of λmyc IμHABCL6 mice bearing a floxed YY1 locus by crossing with γ1-CRE mice. Development of B cell lymphoma in YY1-conditional null λmyc IμHABCL6 mice will be assessed along with survival, tumor growth, and tumor sub-types as above. We predict reduced B cell lymphomagenesis, extended latency time, and increased survival in the absence of YY1.
2a. Breed λmyc IμHABCL6 mice onto a yy1f/f γ-CRE background.
Time Frame: months 4-18. 90% completed

2b. Evaluate tumor latency, tumor development by histology and pathology. Beginning 3 months after birth and each month thereafter for 10 months, three mice will be sacrificed each month for evaluation of tumor development. A complete autopsy will be performed on each YY1 overexpressing and vector control mouse. Routine hematoxylin and eosin (H&E) stained slides of spleen, liver, lymph nodes, bone marrow, neoplastic tissue and any other tissues that appear suspect will be reviewed histologically by a board certified veterinary pathologist (Dr. Amy Durham). Germinal center phenotype will be

initially assessed by staining for PNA and CD95
Time Frame: months 8-24. 10% completed

2c. Molecular profiling of tumors. Neoplasms will be isolated surgically and evaluated molecularly for mutations indicative of germinal center phenotype and by RNA microarray analyses for lymphoma subtyping as described above in 1b.
Time Frame: months 8 to 24. 0% completed

2d. Evaluation of tumor aggressiveness. Proliferation potential and tumor aggressiveness will be assessed as described above in 1d.
Time Frame: months 6 to 24. 0% completed

What was accomplished under these goals?
Our major objective is to determine if YY1 levels impact the level of AID-dependent B cell lymphoma in mice. Specifically, we plan to either over-express YY1 in mice that spontaneously develop B cell lymphoma to determine if YY1 over-expression leads to increased lymphoma, or delete YY1 in the same mice to determine if this results in a reduction in B cell lymphoma.

Our significant results during the past year are below:
1. In task 1 we bred λmyc, IμHABCL6, and λmyc IμHABCL6 mice to generate sufficiently large colonies for our experiments. The mice bred somewhat slowly at first which initially hampered progress, but these difficulties have been solved. Bone marrow from λmyc IμHABCL6 mice was transduced with retrovirus vectors consisting of either vector alone (MigR1) or YY1-expressing vector (MigR1-YY1). Transduced cells were transferred to secondary irradiated recipient mice and allowed to develop. In our preliminary experiments we have found that the YY1-expressing mice appear to become sick earlier than the MigR1-expressing mice. However, our numbers of analyzed mice are too low to be confident of this conclusion. We have had higher than the normal level of death post-transplantation into irradiated recipient mice. We are attempting to reduce this loss by giving the recipient mice water containing antibiotics two weeks prior to transplant rather than just coincident with transplant. The lower number of surviving mice than expected has also hampered our ability to perform histology and to molecularly profile the tumors that develop. However, we anticipate being able to fully accomplish this aim in the coming year.

2. In task 2 we have successfully bred the IμHABCL6 background into a $yy1^{f/f}$ γ-CRE background. This is a very important step that took many generations of breeding during the past year. These mice will enable us to determine whether deletion of the *yy1* gene results in reduced B cell lymphoma. Now that we have these mice in hand, we will be able to expand them out to fully complete the experiments in Task 2. We hypothesize that deletion of YY1 will reduce the frequency and severity of B cell lymphoma. Such results would be very exciting and would indicate that YY1 should be explored as a potential target for treatment of B cell lymphoma.

3. In parallel experiments we have obtained direct evidence that YY1 controls the mutation rate of AID. We looked at AID mutation rates in vivo in two different systems. First, we deleted YY1 in splenic B cells from IgκAID mice that overexpress AID leading to high mutation rates at the Smu region (an AID target site). Deletion of YY1 led to a statistically significant drop in the AID mutation rate at the Smu region, but not at the Taci gene (a non-AID target site) in agreement with our hypothesis (Fig. 1A, below). In a second system, *yy1*$^{f/f}$ mice which have normal AID expression levels, we found that deletion of the *yy1* gene led to a dramatic loss of AID-dependent mutations (Fig. 1B). This confirms our results in IgκAID mice and extends them into a more physiological setting. These results give us a high degree of confidence that our main hypothesis is true. We plan to write these studies for publication within the next several months.

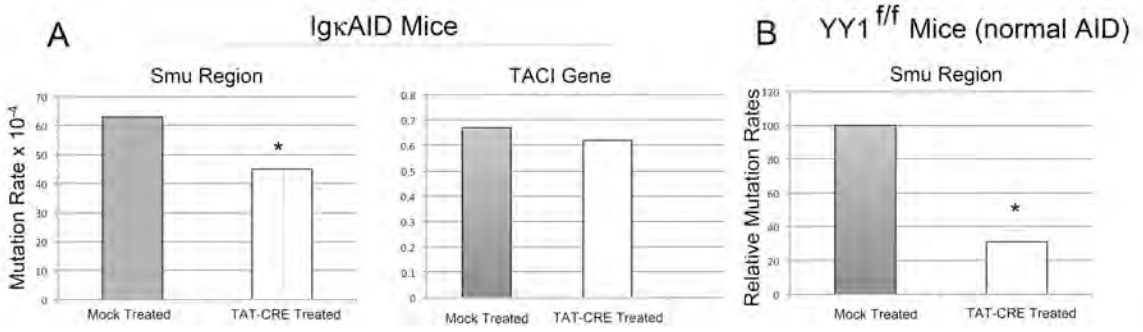

Figure 1. YY1 knock-out reduces AID mutagenic activity at AID-target sites. (A) Knock-out of YY1 reduces AID-mediated mutations at the AID-target Smu region, but not at the AID-nontarget TACI gene. (B) YY1 knock-out reduces AID mutation in mice with normal AID levels at the AID-target Smu region. Asterisks denote statistical significance p<0.002.

What opportunities for training and professional development has the project provided?
Nothing to report.

How were the results disseminated to communities of interest?
Nothing to report. Publications expected in the coming year.

What do you plan to do during the next reporting period to accomplish the goals?
After the initial delays in in our progress, we now believe progress will be more in line with our original expectations. Our plan for the coming year is largely to continue the work as outlined in Tasks 1 and 2. We will transduce bone marrow with YY1 or vector expressing retroviruses, transplant this bone marrow into larger numbers of irradiated recipient hosts, and will monitor tumor formation by histology and pathology, as well as by molecular profiling. Select samples will be evaluated for tumor aggressiveness.

Simultaneously we will evaluate the impact of YY1 knock-out on the latency, incidence, and aggressiveness of B cell lymphoma. Conditional YY1 knock-out mice will be evaluated for tumor development by histology and pathology, by molecular profiling, and by tumor aggressiveness.

We anticipate that our experiments will either support or disprove our hypothesis that YY1 expression levels regulate AID mutagenic activity, and as a consequence, the level of B cell lymphoma.

4. IMPACT

What was the impact on the development of the principle discipline of the project?

Our results have shown that reduction of YY1 levels impacts the rate of AID mutation in vivo. This is a very significant result and has a direct impact on the discipline of AID-mediated mutagenesis. In the coming year we hope to determine if this YY1-regulated change in AID mutation rates causes a corresponding change in the level and aggressiveness of AID-dependent B cell lymphomas.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS

Changes in approach and reasons for change

No significant changes in approach.

Actual or anticipated problems or delays and actions or plans to resolve them.

There were initial delays due to slow breeding by the mice. But this has been resolved and the mice are breeding well now. We've also had higher than usual mortality of mice after bone marrow transplant. We believe that problem has now been largely resolved and anticipate no further delays.

Changes that had a significant impact on expenditures

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents.

Nothing to report

6. PRODUCTS

Publications, conference papers, and presentations

We anticipate that three manuscripts will be submitted for publication in the coming year. The first will show the impact of YY1 on AID mutagenic activity. The second will show the impact of YY1 on germinal center biology. The third will focus on the impact of YY1 over-expression or deletion on B cell lymphoma.

Websites or other internet sites
Nothing to report

Technologies or techniques
Nothing to report

Inventions, patent applications, and/or licenses
Nothing to report

Other products
Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals worked on the project?

Name	Michael Atchison
Role on Project	PI
Nearest person month worked	2
Contribution to project	Overall supervision and direction
Funding support	NIH R01 AI079002, NIH GM111384, and Departmental sources

Name	Amy Durham
Role on Project	Pathologist
Nearest person month worked	1
Contribution to project	Pathology analyses
Funding support	Departmental sources

Name	Anupam Banerjee
Role on Project	Research Associate
Nearest person month worked	8
Contribution to project	Bone marrow transplants
Funding support	NIH R01 AI097590

Name	Parul Mehra
Role on Project	Research Associate
Nearest person month worked	4
Contribution to project	Generation of virus, bone marrow transplants
Funding support	NIH R01 AI07002 and R01 GM111384

Has there been a change in the active other support of the PD/PI or senior/key personnel since the last reporting period?
Nothing to report

What other organizations were involved as partners
Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

Nothing to report

9. APPENDICES

Nothing to report